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MicroRNA Affymetrix microarray probe labeling from ocular fluids

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Protocol status: Working

We use this protocol and it's working

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Abstract

Sample collection, isolation of miRNAs, and quality control

Aqueous and vitreous humor, as well as plasma samples, have been collected from Diabetes mellitus (DM) and control patients during the standard-of-the-care eye surgery. Samples of 100-200ul were collected, aliquoted in 100ul aliquots, frozen on dry ice immediately and stored at -80°C. RNA was isolated using Exiqons' modification of Qiagen's microRNeasy kit. Quantification and quality check of isolated miRNAs was performed on BioAnalyzer (Agilent) with Small RNA microfluidics Chips.

Affymetrix microarray probe labeling

Probe labeling was done from 10 ng of aqueous, 20ng of vitreous and 20ng of plasma miRNA samples from a total of 24 aqueous, 24 vitreous and 25 plasma samples using FlashTag (Affymetrix) procedure. Labeled probes were hybridized to Affymetrix miRNA Array 3.0 in UC Davis Genome Center Microarray Core Facility using the standard procedure (Affymetrix, Santa Clara, CA). The total data set included 73 Affymetrix miRNA 3.0 microarrays. Upon scanning of the hybridized and washed Chips, data was obtained in a set of Affymetrix data files (.cel, .arr, .jpg).

